Alpha-Synuclein ELISA Kit Protocol

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Abstract

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Guidelines

Kit Components

- Capture Antibody Coated Plate* - 1 plate
- a-Synuclein Standard - (1) 25µg vial
- 5X Wash Buffer - 250mL
- 2X Reagent Diluent - 32mL
- Biotinylated Primary Antibody - 25µL
- Streptavidin-HRP - 25µL
- Chemiluminescent Substrate A - 6mL
- Chemiluminescent Substrate B - 6mL
- Plate Sealer - 3

** The strips in this plate are not readily removable; it is intended to be used as a whole plate. Attempting to remove the strips will result in damage for which Biolegend is not responsible.*

Storage: Store kit at 2-8oC. We suggest storing the a-Synuclein Standard component only at ≤-70oC if you do not intend to use the kit within 2 weeks of receipt.

TABLE 1. Preparation of Standard Intermediates

Tube Number	Volume of Standard	Volume of 1X Reagent Diluent (µL)	Final Concentration (ng/mL)
Intermediate #1	10µL of reconstituted standard	990	5,000
Intermediate #2	10µL of intermediate #1	990	50

TABLE 2. Preparation of Standard Curve

Tube Number	Volume of Standard (µL)	Volume of 1X Reagent Diluent (µL)	Final Concentration (pg/mL)
1	40µL intermediate #2	1280	1500
2	550 µL of #1	825	600
3	550 µL of #2	825	240
4	550 µL of #3	825	96.0
5	550 µL of #4	825	38.4
6	550 µL of #5	825	15.4
7	550 µL of #6	825	6.1
8	0	825	0

TABLE 3.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 1	Sample 1	Sample 1							
B	Std 2	Std 2	Std 2	Sample 2	Sample 2							
C	Std 3	Std 3	Std 3	Sample 3	Sample 3							
D	Std 4	Std 4	Std 4	Sample 4	Sample 4							
E	Std 5	Std 5	Std 5	Sample 5	Sample 5							
F	Std 6	Std 6	Std 6	Sample 6	Sample 6							
G	Std 7	Std 7	Std 7	Sample 7	Sample 7							
H	zero	zero	zero	Sample 8	Sample 8							

TABLE 4.

Tube Name	Volume Streptavidin-HRP stock (mL)	Volume of 1X Reagent Diluent (mL)	Dilution Factor
HRP Intermediate	0.01	0.990	100
Diluted Streptavidin HRP	0.150 (intermediate)	22.35	150

Suggested Settings for Biotek Synergy2 Plate Reader*

Function	Setting
Detection Method	Luminescence
Read Type	Endpoint
Integration Time	1 second
Emission	Hole
Optics Position	Top
Sensitivity	110

*Settings for other plate readers unknown.

Consult your owners manual prior to starting the assay to determine the optimal settings for your instrument

Protocol

Preparation of 1X Wash Buffer

Step 1.

Notes: Allow kit components to be brought to room temperature before use.

Label a 1L bottle as “1X Wash Buffer”.

Preparation of 1X Wash Buffer

Step 2.

Dilute 5X Wash Buffer 1:5 using lab grade water* and mix *well.*

**Note:* Lab grade filtered water such as injection grade, cell culture grade, Reverse Osmosis De-Ionization
(RODI).

Preparation of 1X Reagent Diluent

Step 3.

Label an appropriate sized bottle as “1X Reagent Diluent”.

Preparation of 1X Reagent Diluent

Step 4.

Dilute 2X Reagent Diluent to 1X by adding 30mL of 2X Reagent Diluent to 30mL of lab grade water in the bottle labeled “1X Reagent Diluent”.

Preparation of 1X Reagent Diluent

Step 5.

Mix well by vortex.

Preparation of Standard Intermediates (Refer to Table 1)

Step 6.

Reconstitute lyophilized standard with 50µL of lab grade water and mix well by vortex. The concentration after reconstitution will be 500µg/mL.

Preparation of Standard Intermediates (Refer to Table 1)

Step 7.

Label (2) 1.5mL microcentrifuge tubes as intermediate #1 & intermediate #2.

Preparation of Standard Intermediates (Refer to Table 1)

Step 8.

Aliquot 990µL of 1X Reagent Diluent into intermediate #1 & intermediate #2 tubes.

Preparation of Standard Intermediates (Refer to Table 1)

Step 9.

Remove 10µL from the vial of reconstituted standard and add to 990µL of 1X Reagent Diluent in intermediate tube #1. **Mix well by vortex.**

Preparation of Standard Intermediates (Refer to Table 1)

Step 10.

Remove 10µL from intermediate #1 and add to 990µL of 1X Reagent Diluent in intermediate #2 tube. **Mix well by vortex.**

Preparation of Standard Intermediates (Refer to Table 1)

Step 11.

The final concentration of intermediate tube #2 will be 50ng/mL.

Preparation of Standard Curve (Refer to Table 2)

Step 12.

Label (8) 1.5mL microcentrifuge tubes as #1-8.

Preparation of Standard Curve (Refer to Table 2)

Step 13.

Add 1280µL of 1X Reagent Diluent to tube #1 and 825µL of 1X Reagent Diluent to tubes #2-8.

Preparation of Standard Curve (Refer to Table 2)

Step 14.

Remove 40µL from intermediate #2 and add to 1280µL of 1X Reagent Diluent in tube #1.

Preparation of Standard Curve (Refer to Table 2)

Step 15.

Mix well by vortex (this will be the top point of the standard curve, 1500pg/mL).

Preparation of Standard Curve (Refer to Table 2)

Step 16.

Remove 550µL from tube #1 and add to 825µL of 1X Reagent Diluent in tube #2.

Preparation of Standard Curve (Refer to Table 2)

Step 17.

Mix well by vortex.

Preparation of Standard Curve (Refer to Table 2)

Step 18.

Continue making 2.5 fold serial dilutions by adding 550µL of the previous dilution to 825µL of 1X Reagent Diluent in tubes #3-7. Mix well by vortex between each dilution.

Note: Tube #8 will be the zero or blank sample and should only contain 1X Reagent Diluent

Sample Preparation

Step 19.

Dilute samples in 1X Reagent Diluent. Mix each dilution by vortexing 3 X 2 seconds.

Sample Preparation

Step 20.

Run samples in duplicate or triplicate.

Note: It is good practice to run at least 2 dilutions for each sample to ensure one of the dilutions falls within the linear range of the standard curve. We recommend a minimum dilution of 1:10.

Note: Dilution factors will vary based on the sample matrix of your experiments. We recommend running a subset of your samples in the kit to determine optimal dilutions and evaluate any matrix effects. Failure to do this type of optimization could result in inconclusive data, for which Biolegend is not responsible.

Running the Assay - Day 1

Step 21.

Remove the plate from the foil pouch.

Running the Assay - Day 1

Step 22.

Add 300µL/well of 1X Wash Buffer.

Running the Assay - Day 1

Step 23.

Dump out wash buffer and pat dry.

Running the Assay - Day 1

Step 24.

Repeat 3 more times for a total of 4 washes.

Running the Assay - Day 1

Step 25.

Add 200µL of each standard to the plate in duplicate or triplicate. Follow the plate layout below.

Note: The volumes in Table 2 are sufficient for running the standard curve in triplicate.

Running the Assay - Day 1

Step 26.

Add 200µL of each sample dilution to the plate.

Running the Assay - Day 1

Step 27.

Cover the plate with the plate sealer provided.

Running the Assay - Day 1

Step 28.

Incubate overnight at 2-8^{o}C, while shaking.

Running the Assay - Day 2

Step 29.

Notes: Allow kit components to be brought to room temperature before use.

Preparation of Biotinylated Primary Antibody

i. Label a 15mL centrifuge tube as “Biotinylated Primary Antibody”

ii. Dilute the biotinylated primary antibody by adding 6µL* of the Biotinylated Primary

antibody stock to 6mL of 1X Reagent Diluent in the 15mL tube labeled “Biotinylated Primary Antibody”

*_Note:_ A quick spin in a centrifuge is suggested prior to pipetting to ensure liquid is at the bottom of the vessel

.
iii. Mix well by vortex

Running the Assay - Day 2

Step 30.

Remove plate from refrigerator and dump contents.

Running the Assay - Day 2

Step 31.

Add 300µL/well of 1X Wash Buffer.

Running the Assay - Day 2

Step 32.

Dump out wash buffer and pat dry.

Running the Assay - Day 2

Step 33.

Repeat 3 more times for a total of 4 washes.

Running the Assay - Day 2

Step 34.

Add 50µL/well of the Biotinylated Primary Antibody to the plate (prepared above).

Running the Assay - Day 2

Step 35.

Cover and incubate for 2 hours at room temperature.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 36.

Label a 1.5mL microcentrifuge tube as “HRP Intermediate”.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 37.

Label a 50mL tube as “Diluted Streptavidin HRP”.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 38.

Make the “HRP Intermediate” by adding 10µL* of Streptavidin-HRP stock to 990µL of 1X Reagent Diluent in the 1.5mL microcentrifuge tube labeled “HRP Intermediate”.

**Note*: A quick spin in a centrifuge is suggested prior to pipetting to ensure liquid is at the bottom of the vessel.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 39.

Mix well by vortex.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 40.

Remove 150µL from the HRP Intermediate tube and add to 22.35mL of 1X Reagent Diluent in the 50mL tube labeled “Diluted Streptavidin HRP”.

Running the Assay - Day 2 - Preparation of Streptavidin HRP (Refer to Table 4)

Step 41.

Mix well by vortex.

Running the Assay - Day 2

Step 42.

Remove plate from incubation and dump contents.

Running the Assay - Day 2

Step 43.

Add 300µL/well of 1X Wash Buffer.

Running the Assay - Day 2

Step 44.

Dump out wash buffer and pat dry.

Running the Assay - Day 2

Step 45.

Repeat 3 more times for a total of 4 washes.

Running the Assay - Day 2

Step 46.

Add 200µL/well of Diluted Streptavidin HRP to the plate (prepared above).

Running the Assay - Day 2

Step 47.

Cover and incubate for 1 hour at room temperature.

Running the Assay - Day 2

Step 48.

Remove plate from incubation and dump contents.

Running the Assay - Day 2

Step 49.

Add 300µL/well of 1X Wash Buffer.

Running the Assay - Day 2

Step 50.

Dump out wash buffer and pat dry.

Running the Assay - Day 2

Step 51.

Repeat 3 more times for a total of 4 washes.

Running the Assay - Day 2

Step 52.

Mix chemiluminescent substrates for use:

i. Add 5.5mL of substrate A to a 15mL centrifuge tube.

ii. Add 5.5mL of substrate B to the same 15mL centrifuge tube.

iii. Mix well by vortex.

Running the Assay - Day 2

Step 53.

Add 100µL of mixed substrate per well.

Note: If reading multiple plates add substrate one plate at a time; do not add substrate to all plates at the same time. Add substrate to each plate immediately before reading.

Running the Assay - Day 2

Step 54.

Shake plate on either a plate shaker or using the shaking mechanism within the platereader for 10-15 seconds.

Running the Assay - Day 2

Step 55.

Read plate immediately.

Note: The recommended luminometer settings are to read at a mid-range sensitivity level for 1 second
per well. These settings will vary between plate reader manufacturers, please consult your owner's manual prior to performing this assay

Step 56.

See Biolegend.com for additional data